

The cellular architecture of multiple myeloma

Carolina Vicente-Dueñas,^{1,2,*} Isabel Romero-Camarero,^{1,2} Francisco Javier García-Criado,^{2,3} César Cobaleda⁴
and Isidro Sánchez-García^{1,2,*}

¹Experimental Therapeutics and Translational Oncology Program; Instituto de Biología Molecular y Celular del Cáncer (IBMCC); CSIC/ University of Salamanca; Salamanca, Spain; ²Institute of Biomedical Research of Salamanca (IBSAL); Salamanca, Spain; ³Departamento de Cirugía; Universidad de Salamanca; Salamanca, Spain; ⁴Centro de Biología Molecular Severo Ochoa; CSIC/Universidad Autónoma de Madrid; Madrid, Spain

Multiple myeloma (MM) is a human cancer of malignant plasma cells. Recent genomic studies have started to catalog all the genetic alterations in selected panels of MM samples, and, within a short period of time, the large majority of MM-associated genetic and transcriptional alterations will have been identified. These recent insights, on one hand, indicate that MMs can harbor extensive clonal heterogeneity, and, on the other hand, they will help to reconstruct the molecular ontogeny of the disease's evolution (i.e., the clonal architecture of each individual MM). However, a pending challenge is to delineate the stages of hematopoietic differentiation at which the individual genetic alterations arise, or, in other words, to elucidate the cellular architecture of MM.

In the classical view of the initiation and progression of MM, an initiating genetic alteration is required to immortalize a plasma cell (Fig. 1A). Such cell is then destined to acquire additional genetics hits over time. The acquisition of additional hits further deregulates the behavior of the plasma cells, thus leading to the clinically recognized features of MM, including the lytic bone lesions. In this classical model, MM is therefore a disease arising from mutations occurring in an already differentiated plasma cell. However, in the last years it has been shown that cellular heterogeneity is also a feature of human MM. Indeed, by studying the engraftment of primary MM specimens in immunodeficient mice, it was found that tumor plasma cells were unable of engraftment following intravenous injection,¹ suggesting that the MM-propagating/maintaining cell

was not a tumor plasma cell. Therefore, an essential gap that must be covered in order to define the cellular architecture of MM is the identification of the nature of the MM cell of origin. In fact, in spite of the assumptions of the above-mentioned classical view of MM origin, very little is still known about the cancer cell of origin from which MM arises and about how the initial genetic lesion(s) regulate this essential step. These initial genetic lesions have always been detected in differentiated tumoral plasma cells, although the presence of a human myeloma stem cell was postulated many years ago.² It is true that the patient's hematopoietic progenitor and stem cells (HS/PCs) seem not to show any of the initiating genetic alterations detected in tumor plasma cells; however, it is also true that these alterations would be very difficult to detect if the frequency of these putative stem cells harboring the MM-inducing genetic lesion was low. Therefore, due to this purely statistical reason, experimental results would seem to suggest that the MM cell of origin is not a stem/progenitor cell. However, until now, all the experiments in which the expression of human MM-linked oncogenes is targeted to the mouse differentiated B cell compartment have failed to reproduce the human disease in mice. Therefore, it is potentially possible that in human patients, the occurrence of MM-associated oncogenic alterations might happen in the HS/PCs compartment, and this cell of origin adopts/acquires afterwards a MM cell fate as a consequence of the oncogene activity.

To elucidate if MM is a stem cell-driven tissue, we initially focused on the effects of the *MafB* oncogene, since the MAF family

of proteins seems to be implicated in 50% of human MM. In consequence, we have developed mice in which we limited *MafB* expression to the mouse HS/PCs Sca1⁺ cells.³ In human MM and in most animal models of cancer,⁴ the oncogenic alterations are expressed in all the cellular types that compose the tumoral tissue, from the MM stem cell to the terminal differentiated plasma cells. In our stem cell-driven Sca1-MafB model, the expression of *MafB* is restricted to the HS/PCs compartment, but this restricted expression pattern is nevertheless capable of generating a full-blown MM with all its differentiated cellular components, in such a way that Sca1-MafB mice share both major and minor diagnostic criteria with the human pathology. The demonstration that MM development can be established in mice by expressing *MafB* only in Sca1⁺ cells implies that *MafB* can impose a gene regulatory state in these stem cells that somehow persists during hematopoiesis, and that can program a tumor plasma cell phenotype symptomatic of MM. This is an observation that seems to apply to other cancer-initiating gene defects.^{5–10} In the oncogenic reprogramming model here presented, the reprogrammed Sca1⁺ population can nevertheless complete a multi-stage differentiation pathway involving an initial commitment to the B cell lineage and a subsequent differentiation to tumor plasma cells.³ This model of MM is very informative with respect to the fact that the oncogenic mutations can have different roles in MM stem cells vs. differentiated tumor plasma cells. Thus, genetically distinct MM stem cell subclones may exist on top of each MM tumor mass, revealing an even higher complex cellular

*Correspondence to: Carolina Vicente-Dueñas and Isidro Sánchez-García; Email: cvd@usal.es and isg@usal.es

Submitted: 08/28/12; Accepted: 08/29/12

<http://dx.doi.org/10.4161/cc.22178>

Comment on: Vicente-Dueñas C, et al. EMBO J 2012 31:3704–17; PMID:22903061; <http://dx.doi.org/10.1038/emboj.2012.227>.

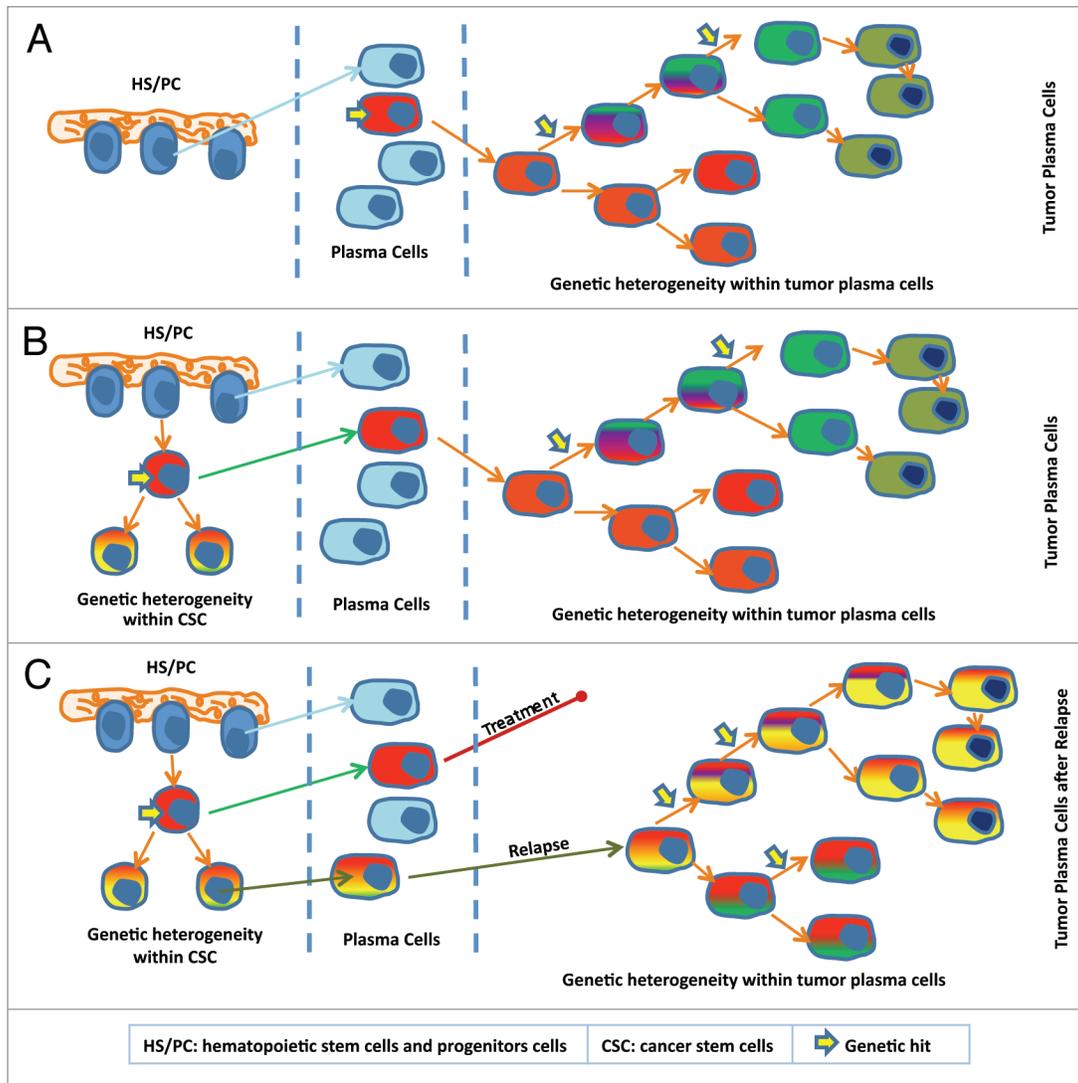


Figure 1. Cellular architecture and multiple myeloma (MM) development. (A) Current working model for the development of MM in humans. In the current view of the initiation and progression of MM, an initiating hit is required to immortalize a plasma cell. Such cells are then destined to acquire additional genetics hits over time. The acquisition of additional hits further deregulates the behavior of the plasma cells, thus leading to a subclonal genetic heterogeneity within tumor plasma cells. Specific subclones of tumor plasma cells may contribute to the initiation, treatment resistance or relapse. (B and C) A model by which ectopic expression of MafB reprogrammes HS/PCs into tumor plasma cells. This new model of MM development is based on the idea that initiating hits take place within stem/progenitor cells. These genetic or epigenetic changes would not interfere with normal B cell development, but become active in the process of terminal plasma cell differentiation, leading to the appearance of MM. According to this model, MM is the result of a cell reprogramming process, where genetically distinct MM stem cell subclones may exist on top of each tumor MM mass, revealing a highly complex cellular composition of MM. According to this model, the cellular types that compose the tumoral tissue include MM stem cells close to the terminal differentiated tumor plasma cells (B). The genetic heterogeneity can affect any cellular type of the MM tissue and any of the specific subclones of MM stem cells or tumor plasma cells may contribute to the different steps of the MM evolution before (B) and after treatment (C).

composition of MM than initially thought (Fig. 1B).

In order to gain even more insight into the mechanism by which MafB can induce the reprogramming of stem cells into tumor plasma cells, we have also generated *in vivo* genome-scale maps of DNA methylation from both stem cells and mature B cells from Sca1-MafB mice.³ We found that a substantial number of

CpG islands and promoters are specifically hypermethylated or hypomethylated in the stem cells of Sca1-MafB mice, setting a pattern inherited throughout B cell development. Thus, the results presented in our study demonstrate a novel molecular mechanism involved in tumor initiation, by showing that HS/PCs can be epigenetically reprogrammed to give rise to terminally differentiated tumor plasma

cells by MafB.³ To our knowledge, these results represent the most convincing evidence to date that MM development can arise and be driven by a tumor cell fate change within the stem cells. These findings represent a novel paradigm in MM biology and will revolutionize the current modeling of MM tumorigenesis and progression and are likely to have profound therapeutic implications (Fig. 1C).

References

1. Matsui W, et al. *Blood* 2004; 103:2332-6; PMID:14630803; <http://dx.doi.org/10.1182/blood-2003-09-3064>.
2. Hamburger A, et al. *J Clin Invest* 1977; 60:846-54; PMID:302265; <http://dx.doi.org/10.1172/JCI108839>.
3. Vicente-Dueñas C, et al. *EMBO J* 2012; PMID:22903061; <http://dx.doi.org/10.1038/emboj.2012.227>.
4. Vicente-Dueñas C, et al. *Dis Model Mech* 2010; 3:149-55; PMID:20212083; <http://dx.doi.org/10.1242/dmm.002774>.
5. Pérez-Caro M, et al. *EMBO J* 2009; 28:8-20; PMID:19037256; <http://dx.doi.org/10.1038/emboj.2008.253>.
6. Vicente-Dueñas C, et al. *Proc Natl Acad Sci USA* 2012; 109:10534-9; PMID:22689981; <http://dx.doi.org/10.1073/pnas.1204127109>.
7. Vicente-Dueñas C, et al. *Cell Cycle* 2009; 8:1314-8; PMID:19279406; <http://dx.doi.org/10.4161/cc.8.9.8217>.
8. Vicente-Dueñas C, et al. *Oncotarget* 2012; 3:261-6; PMID:22408137.
9. Vicente-Dueñas C, et al. *Aging (Albany NY)* 2010; 2:908-13; PMID:21164221.
10. Chen J, et al. *Nature* 2012; 488:522-6; <http://dx.doi.org/10.1038/nature11287>; PMID:22854781.